The utilization of non-Saccharomyces Yeast for organoleptic properties and Bioprotection
Chr-Hansen
Hentie Swiegers
Ad van Etten

Viniflora® Non-Saccharomyces yeasts
Bio-science to protect & create
Chr-Hansen’s expertise is in fermentation management

- 1980  Introduced Viniflora™; first direct inoculation concept for MLF
- 2004  First to introduce non-sacch/sacch yeast blends
- 2006  Introduced FroZen direct inoculation MLF cultures a cost efficient solution for large wineries.
- 2009  Prelude™, the 1st pure Torulaspora delbruecki
- 2010  Frootzen™, the 1st pure Pichia kluveri
- 2011  Concerto™, the 1st pure strain of Kluyveromyces thermotolerans
- 2011  CiNe™, 1st citrate negative malolactic culture
- 2012  Freasy™, a frozen MLF concept that can be kept for 3 months at -18C
- 2014  NOVA™, 1st MLF concept for low/no sulfite wine making process (US approval pending)
- 2014  Biggest single customer order for direct inoculation MLF cultures
- 2014  Full innovation pipeline to stay ahead
MICROWINE EU GRANT

FROST & SULLIVAN
2014 AWARD
NEW PRODUCT INNOVATION LEADERSHIP
MFC* sequence in winemaking

Population over time in days then weeks

Alcoholic fermentation

Non-Saccharomyces spp.

Saccharomyces spp.

Malolactic fermentation

Oenococcus oeni / Lactobacillus spp.

* MFC: Microbial Food Cultures
Where a high concentration ① of identified and living microorganisms is used - instead of preservatives -, to avoid the development ② of spoilage microorganisms (yeasts, molds, bacteria) that could degrade ③ product microbial quality, product organoleptic characteristics and by that spoil or render the product unsafe.

① the use of *Saccharomyces cerevisiae* starter cultures to kick-off alcoholic fermentation and produce ethanol to reduce the risk of spoilage organisms developing in the must (e.g. spontaneous yeast)

② the inoculation of wine with known DI *Oenococcus oeni* strains to manage MLF and suppress undesirable Lactic Acid Bacteria like Pediococci

③ the use of pure strain *Torulaspora delbrueckii* at the pre-fermentation maceration phase, outcompeting wild yeasts and or molds
Bio-protection in winemaking:

**Competitive exclusion**
- The culture or the blend of cultures selected can overcome other species/strain of microorganisms growth in specific conditions

- The effect is linked to both:
  1. the strain(s) selected and therefore its physiology/production method
  2. the concentration of active cells after inoculation (until a specific limit of action in cfu/ml)
  3. the growth rate of this strain

Therefore knowledge, expertise in microbiology and production of cultures is a key success factor

**Compounds production**
- The culture or the blend of cultures selected are able to produce specific compounds limiting the development of technical or pathogenic contaminants

- The effect is strain dependent and can be linked to production of:
  1. ethanol
  2. organic acids
  3. peptides: mycocines, killer factors, bacteriocines
  4. ...

**Jameson effect**
Minority population decelerates when the majority or the total population reaches its maximum (competition for a common limiting resource or for space?)

Most numerous bacteria if well adapted, inhibit the growth of the other via physical interactions
You can not reach more than 1E+08/1E+09 cfu/g in solid food
Jameson effect

Bacterial concentration (log cfu/g)

Protective culture

Contaminants

Contaminants (molds, yeasts, bacteria) + protective culture

Simultaneous deceleration (Lag and $\mu_{\text{max}}$ similar)
Bio-protection to create:

**Winery/Process benefits**

- **Grape Potential**
  - Minimise risk of defects and reduce downgrading related to fermentation mismanagement
  - Bring out true wine potential through protection and release of Flavors; Mouth-feel and Color

- **Fermentation management**
  - Control AF and MLF processes to manage timing
  - Secure biosafety of berries, musts or wines to prevent contamination
  - Add full ingredient traceability to fermentation processes

**Consumer benefits**

- **Cleaner Recipe**
  - Less or no SO2 in winemaking or in final wines
  - Less or no preservatives (ascorbic acid/sorbates...)
  - Reduced negative compound production/off flavours

- **Brand love**
  - Consistency (not uniformity)
  - Sustainability
  - Health

**Build margins**

- **Create value**
  - Biogenic amine control
  - Health
  - Sustainability
  - Consistency (not uniformity)
Reduce sulfites in winemaking
Invent new wines for new customers with Viniflora®
example of customer activities (Italy targeting Northern Europe)

Low histamine wine
MLF managed with Viniflora® CH16

http://www.e-magin.se/v5/viewer/files/viewer_s.aspx?gKey=jnj06xmr&gInitPage=33
Incremental quality improvements

Quality

Yesterday

Today

Tomorrow

Yield control  Comm Yeast  Temperature  MLF  Non-Sacch  Sustainability

biosafety  Health

Process control  Waste

Speed  CO2

Functionality

Security

Economy

Time
Viniflora® Non-Saccharomyces yeasts
A Chr. Hansen concept for Bio-protection & more

P. kluyveri
YGC 25C

IMPROVE MUST IMPROVE
MUST IMPROVE MUST IMPROVE
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Application history of non-Saccharomyces yeast in the wine

- *Kluyveromyces thermotolerans* (*S. veronae*) promoted by Brice Rankine in the late 1940’s and onwards (AWRI 173)
- *Torulaspora delbrueckii* (*S. rosei*) deposited by Rainer Eschenbruck in 1975 and promoted for sweet wines (AWRI 1034)
- Blend of *K. thermotolerans*, *T. delbrueckii* and *S. cerevisiae* ADY launched in 2007/2008
- Single strain *T. delbrueckii* ADY launched in 2009
- Single strain *Pichia kluyveri* frozen yeast launched in 2010
- Single strain *K. thermotolerans* ADY launched in 2012
Non-Saccharomyces in the ‘wine literature’

# publications

2005-2012

- torulaspora
- kluyveromyces
- pichia
- metschnikowia
- schizosaccharomyces
- Hanseniaspora
- Non-saccharomyces

Year

2005 2006 2007 2008 2009 2010 2011 2012
The Microbial Population Dynamics in Wine

Non-Sacc’s proliferate at early stages

Saccharomyces takes over, after starting from a low conc.

The ecology of micro-organisms during winemaking is very complex. The following species of yeast can be found:

- **Brettanomyces / Dekkera**
- **Candida**
- **Cryptococcus**
- **Debaromyces**
- **Hanseniaspora / Kloekera**
- **Hansenula**
- **Kluyveromyces**
- **Torulaspora**
- **Saccharomycodes**
- **Schizosaccharomyces**
- **Zygosaccharomyces**
- **Metschnikowia**
- **Pichia**
- **Rhodotorula**
What properties do non-Saccharomyces yeast develop in wine?
Non-Sacch yeasts: wine characteristics impacted per type

1 species among wine NSAC yeast has shown the highest potential: *Pichia kluyveri*

1 species among wine NSAC yeast has shown the highest potential: *Lachancea* thermotolerans

* formerly *Kluyveromyces thermotolerans*

1 species among wine NSAC yeast has shown the highest potential: *Torulaspora delbrueckii*

Flavors

- Precursors conversion
- Metabolism differences

Acid balance

- Organic acids production/reduction

Mouth-feel

- Polysaccharides
- Mannoproteins

* Concerto™
* Prelude™
* FrootZen™
FrootZen™: the magic of direct inoculation
Sauvignon blanc, 2010, New Zealand

Volatile thiols (ng / l)

- Control
- Pk Co-inoc
- Pk Seq

3MH
3MHA

CHR HANSEN
Improving food & health
Thiols analysis on Sauvignon blanc

<table>
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<tr>
<th>Sample</th>
<th>3-MH (µg/l)</th>
<th>3MHA (µg/l)</th>
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<tbody>
<tr>
<td>Cuve 24 – control</td>
<td>240</td>
<td>33</td>
</tr>
<tr>
<td>Cuve 25 - Frootzen™</td>
<td>324</td>
<td>42</td>
</tr>
</tbody>
</table>
Sensory profile improvement with FrootZen™ white wine example (Sauvignon blanc)

Range of flavours mentioned by external trained/non professional jury
Loire Valley wine (Sancerre - Sauvignon blanc)
Data: In senso veritas, Feb. 2013

Tropical fruit notes
Floral notes
Sensory profile improvement with FrootZen™ red wine example (Pinot Noir)

Figure 3 Descriptive sensory evaluation on the Pinot noir. Red bars are for the Pinot noir with Viniflora FrootZen and blue bars are for the control Pinot noir (without FrootZen).

Figure 1 Concentration of 3-mercapto-2-hexanone (3MH) in Pinot noir from Montes 2013 with and without Viniflora FrootZen.
Viniflora® FrootZen™: main applications

Fruit forward wines:
Chardonnay, Viognier, Riesling
Pinot gris, S. blanc, Chenin…

Pinot Noir, Syrah
Merlot, Cabernet-Sauvignon, Grenache N.

Fruit forward rosé wines
Viniflora® PRELUDE™

The perfect choice to get ‘wild ferment’ benefits without the risks.
Torulaspora delbrueckii - effect on palate-weight: high production of polysaccharides

Table 3
Analytical profile of wines obtained with the mixed starter cultures used.

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<td>97 ± 10²</td>
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<td>S. cerevisiae 10⁵</td>
<td>13.87 ± 0.06a</td>
<td>3.16 ± 0.05a</td>
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<td>6.84 ± 0.02a</td>
<td>0.44 ± 0.05a</td>
<td>7.18 ± 1.30b</td>
<td>140 ± 42²</td>
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<td>C. zemplinina + S. cerevisiae 10³</td>
<td>13.64 ± 0.04b</td>
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<td>7.95 ± 1.28b</td>
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<td>L. thermotolerans + S. cerevisiae 10⁷</td>
<td>13.80 ± 0.02a</td>
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<td>13.65 ± 0.19b</td>
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<td>6.64 ± 0.37³</td>
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Source: Comitini, F., et al., Selected non-Saccharomyces wine yeasts in controlled multistarter f..., Food Microbiology (2011)
Viniflora® Prelude™ - *Torulaspora delbrueckii*
example of volatile acidity reduction & fatty acids reduction

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<tr>
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<th>Acetic acid g/l</th>
<th>Glycerol g/l</th>
<th>Esters mg/l</th>
<th>Fatty acids (C6,8,10,12) mg/l</th>
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<td><em>S. cerevisiae</em> (EC1118)</td>
<td>0.21</td>
<td>6.7</td>
<td>6.3</td>
<td>14.5</td>
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<td><em>T. delbrueckii</em> (Prelude™) + <em>S. cerevisiae</em> (EC1118)</td>
<td>0.06</td>
<td>6.9</td>
<td>7.3</td>
<td>3.5</td>
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p.t. ~ 20 mg/L

The primary reason for the decrease in volatile acidity and fatty acids is the high tolerance of Td yeasts for high sugar substrates, they are less stressed by their environment than *Saccharomyces* spp.
Sensory effect of Prelude

Comparison between control in purple (S. cerevisiae) and experiment in blue (PRELUDE™ + S. cerevisiae) demonstrating the higher flavor intensity with PRELUDE™ in wines from Sauvignon Blanc. Experiment and grading achieved externally, in France by IFV Sud Ouest, V´innopôle 2010.
PRELUDE™ : main applications

- Woody whites
  - Chardonnay

- Pinot gris

- Merlot, Cabernet-Franc, Cabernet sauvignon
  - Pinot Noir,

- Delicate and low alcohol rose
Viniflora® CONCERTO™

Pure Kluyveromyces thermotolerans

The perfect choice to get ‘wild ferment’ complexity on red wines without the risk.
Concerto™ - *Kluyveromyces thermotolerans* - strain CH456

lactic acid production -natural acidification- ideal for warm climates red or rosé wines

Sc only control

*Kluyveromyces* thermotolerans + Sc

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* : *Kluyveromyces thermotolerans* is also named *Lachancea thermotolerans* (L. thermotolerans)
Ethyl lactate synthesis

Lactic acid + Ethanol ↔ Ethyl lactate + Water

- Lactic acid
- Ethanol
- Ethyl lactate
- Water
Ethyl lactate production

![Strawberries](image.jpg)

- **S. cerevisiae**
- **Prelude**
- **Concerto**
- **Spontaneous**

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</tr>
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<td>Prelude</td>
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</tr>
<tr>
<td>Concerto</td>
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<td>Spontaneous</td>
<td>45</td>
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Non Sacch: Sequential Inoculation, up to 48 hours before *S. cerevisiae*

- **Inoculation of Concerto™**
- **Inoculation with usual standard *S. cerevisiae* yeast**
- **25-35 points up to 6-8 °B**
- **Wine ready for malolactic fermentation with Viniflora® cultures**
CONCERTO™: main winemaking applications

White wines from warm climate areas
   Chardonnay, Semillon, Chenin

Merlot, Cabernet sauvignon
   Grenache N., Syrah, Pinot Noir

Rosés wines from warm areas
60% *Saccharomyces cerevisiae*

20% *Torulaspora delbrueckii*

20% *Kluyveromyces thermotolerans*

Three in one
MELODY™ Chardonnay study by the AWRI

Figure 2. Principal Component Analysis biplot of mean sensory data for Chardonnay wines made with nine different active dry wine yeast products available commercially. Adapted from Curtin et al. 2009.

Harnessing AWRI’s yeast and bacterial research to shape ‘Next-Gen’ Chardonnay Part 2: Influence of yeast, nutritional management and malolactic fermentation

By Christopher D. Curtin, Jennifer R. Belton, Eveline J. Bertowsky, Paul A. Harris, Paul J. Chambers, Markus J. Hiedrich & Isak S. Protzulus
The Australian Wine Research Institute, PO Box 107, Glen Osmond, Adelaide, SA 5064, Australia
Chemical Analysis

Figure 3. Results of volatile thiols analysis for 3MH (A) and 3MHA (B). Samples were analysed at the time of sensory analysis, from two representative fermentation replicates per treatment. Adapted from Curtin et al. 2001.
Consumer Testing

Figure 4. Results of central location test of consumer acceptance for Chardonnay wines made with nine different active dry wine yeast products available commercially. Adapted from Curtin et al. 2009.

*Only Segment 1 showed statistically significant differences

** Segment 1 represents regular wine drinkers
‘Sensory summary’ of impact of non-Saccharomyces yeast
Winemaking:
Sacc: S.G. 101
AF temp: 16 °C
Maceration: 4 hours
MLF: No

Analysis:
RS: 3.5 g/l
Alc: 14.0%
pH 3.5
TA = 4.8g/l
(as tartaric)
**Winemaking:**
Sacc: S.G. 101  
AF temp: 25°C  
Maceration: 6 days  
MLF: Yes

**Analysis:**
RS: 3.0 g/l  
Alc: 13.8%  
pH 3.6  
TA = 4.8g/l  
(as tartaric)
Alcohol production by non-Saccharomyces yeast

Alcohol %

0 5 10 15 20

Merit,
Sacch,
Concerto,
Prelude,
Melody,
Concerto, incr. dosage
Prelude, incr. dosage

0,5% less ethanol
Fermentation kinetics of non-Saccharomyces yeast

- Control
- Merit
- Concerto
- Concerto incr. inoc.
- Prelude
- Prelude incr. inoc.

Gluc / Fruc g/l vs Day
Viniflora® range of yeasts
Thank you for your attention